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Technique

Receptor-Selective Coactivators as Tools to Define the Biology of Specific Receptor-Coactivator Pairs

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Summary

In the absence of specific high-affinity agonists and antagonists, it has been difficult to define the target genes and biological responses attributable to many of the orphan nuclear receptors (ONRs). Indeed, it appears that many members of this receptor superfamily are not regulated by classical small molecules but rather their activity is controlled by interacting cofactors. Motivated by this finding, we have developed an approach to genetically isolate specific receptor-cofactor pairs in cells, allowing us to define the biological responses attributable to each complex. This is accomplished by using combinatorial peptide phage display to engineer the receptor interacting domain of each cofactor such that it interacts selectively with one nuclear receptor. In this study, we describe the customization of PGC-1α and its use to study the biology of the estrogen-related receptor α (ERRα) in cultured liver cells.

Introduction

The ONRs are a subset of the larger nuclear receptor superfamily of transcription factors, for which no physiologically relevant ligands have yet been identified. For some ONRs, it has been possible to develop useful synthetic agonists and antagonists enabling the elucidation of their function in vivo. For others, it has been extremely difficult to develop small molecule regulators, and consequently, progress in defining their functional roles has been impeded. Some of these problems have been mitigated with the advent of siRNA technology, wherein knockdown of receptor expression can be used to substitute for antagonists. However, the constitutive activity of many ONRs is so low that even quantitative knockdown does not provide the dynamic range needed to identify target genes. Thus, there is a clear unmet need for approaches with which to positively and negatively regulate the activity of ONRs in target cells.

We are interested in defining the physiological roles for ERRα, an ONR in the NR3B subfamily. Structurally, the ERRs are most closely related to the estrogen receptors (ERs), and not surprisingly, significant crosstalk between the signaling pathways regulated by these receptors has been observed. However, ERRα also plays ER-independent roles as a regulator of oxidative phosphorylation, fatty acid oxidation, and lipid handling (Huss et al., 2004; Mootha et al., 2004; Sladek et al., 1997). Absent a ligand, however, it has been very difficult to evaluate the relative physiological and pathological importance of the ERRs in estrogen action and in the regulation of metabolism.

Crystallographic analysis has revealed that ERRα is capable of adopting a transcriptionally active conformation in the absence of any obvious electron density in what would be expected to be its ligand binding pocket (Kallen et al., 2004). However, its overexpression in cells results in only modest activation of transcription. Robust activation is observed when ERRα is coexpressed in cells with the coactivator PGC-1α (Huss et al., 2002), suggesting that in place of a small molecule ligand, the transcriptional activity of this and related ONRs may be regulated by cofactor availability. Attempts to develop small molecule regulators of ERRα have yielded only weak antagonists and inverse agonists whose toxicity and receptor crossreactivity limit their use (Willy et al., 2004).

One approach to selectively increase the transcriptional activity of a weakly active receptor is to fuse it to the strong transcriptional activator VP16. However, this approach does not recapitulate the physiological activity of the native receptor, as negatively regulated genes will likely be turned on by this modified protein. Another approach is to activate the receptor with one of its known cofactors. However, because most of the coactivators that interact with ERRα also interact with multiple receptors and unrelated transcription factors, it has proven difficult, even when complemented with siRNA knockdown technology, to study the biology of this receptor by simply manipulating coactivator levels in cells. Thus, we have developed a methodology that has enabled us to selectively and effectively regulate ERRα transcriptional activity. Specifically, we have used combinatorial peptide phage display to engineer the receptor interaction domain of the coactivator PGC-1α such that it interacts with and activates ERRα in a highly selective manner. We validated the use of this customized coactivator by using it to identify ERRα-regulated genes in HepG2 cells, a study that both confirmed the key role of this receptor in oxidative metabolism and uncovered additional pathways in which it is engaged. This technology, we believe, will have broad application, allowing for the development of customized coactivators for other NRs and transcription factors.

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	ERRα	ERRβ	ERRγ	ERα	ERβ	PR-A	GR	AR	RAR	RXR	ROR	TRβ	VDR	LXR	FXR	LRH	PPARγ
SRC1	+	+	+	+++	+	+	++	-	++	++	-	++	++	++	+	nd	nd
L3-02	+++	+++	+++	-	-	+	++	-	+	-	-	-	-	+	-	nd	nd
L3-07	+++	+++	++	-	-	-	-	-	-	-	-	-	-	+	-	nd	nd
L3-09	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L3-12	+++	+++	+++	-	-	+++	++	-	-	++	-	-	-	+	-	nd	nd
L3-28	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L3-37	++++	+++	+++	-	-	++	-	-	++	+++	-	-	-	-	-	nd	nd
L3-49	+++	+++	+++	-	-	++	-	-	++	+++++	-	-	-	-	-	nd	nd
L3-62	+++	+++	++	-	-	-	-/+	-	-	-	-	-	-	+	-	nd	nd
L3-80	+++	++++	+++	-	-	-	-	-	-	-	-	-	-	-/+	-	nd	nd

Figure 1. Evaluation of the Receptor Selectivity of the ERRα-Selective Peptides Identified by Phage Display

The interaction of some ERRα-selective peptides with other nuclear receptors (NRs) was tested in a mammalian-two-hybrid assay by using a VP16-receptor construct and a peptide-Gal4DBD fusion on a 5xGal4-luciferase reporter. ERR peptide activity is defined as a percent of highest ERR activity: −, <10%; +, 10%–25%; ++, 25%–50%; +++, 50%–75%; ++++, 75%–100%; +++++, >100%; and nd, not determined. ERRα-selective peptides with minimal interactions with other NRs are indicated by light or dark shading (light, one to two other NRs; dark, no other NR). Abbreviations: ERR, estrogen receptor-related receptor; ER, estrogen receptor; PR, progesterone receptor; GR, glucocorticoid receptor; AR, androgen receptor; RAR, retinoic acid receptor; ROR, RAR-related orphan receptor ligand binding domain; TR, thyroid receptor; VDR, vitamin D receptor; LXR, liver X receptor; FXR, farnesoid X receptor; LRH, liver receptor homolog 1; and PPARγ, peroxisome proliferator-activated receptor γ.

Results

Generation of an ERR-Selective Coactivator

Given that ERRα tethers PGC-1α to target gene promoters through either of two LXXLL motifs within the coactivator's (CoA) NR-interaction domain (Huss et al., 2002), we hypothesized that it may be possible to alter PGC-1α's NR specificity by reengineering its NR-interaction surface such that it interacts with ERRα at the exclusion of other receptors. In this manner, we believed that it would be possible to functionally isolate this receptor-coactivator pair in target cells and define the biological consequences attributable to this complex.

Previously, we demonstrated that it was possible to use combinatorial phage display of peptide libraries to identify LXXLL-containing peptides that interact in a highly specific manner with the coactivator binding pocket of individual NRs (Chang et al., 1999; Hall et al., 2000). Thus, using purified recombinant ERRα as bait, we screened M13 phage display libraries containing 10^8 different random or semirandom peptides 12–19 amino acids in length. The full details of this procedure and the characterization of the peptides identified are described elsewhere (Chang et al., 1999; Gaillard et al., 2006). In brief, we first identified LXXLL peptides that interacted selectively with ERRα but did not interact with either ERα or β under the same conditions. Peptides that showed a preference for ERR were expressed in cells as a fusion with the GAL4DBD, and their ability to tether different VP16-NR fusions was tested in a mammalian two-hybrid assay. This secondary screen enabled the elimination of those peptides that were (1) capable of interacting with NRs commonly expressed with ERRα in target cells and (2) receptors known to interact with PGC-1α. The results of this analysis, summarized in Figure 1, indicate that some peptides (L3-09, L3-28, and L3-80) are highly selective for the ERRs under the conditions tested.

There are three LXXLL motifs within PGC-1α (Figure 2A, L1–L3); however, only L2 and L3 appear to interact with NRs. Although L2 is the dominant site of interaction for most NRs, we and others have shown that either L2 or L3 is sufficient to allow full ERRα transcriptional activity (Gaillard et al., 2006; Huss et al., 2002; Schreiber et al., 2003). Therefore, we replaced both of the 19 amino acid regions corresponding to each of the L2 and L3 motifs within PGC-1α with sequences corresponding to the L3-09 peptide (PGC-1α 2x9, Figure 2A). When assayed on a 3xERE-TATA-luciferase reporter (Figure 2B), PGC-1α 2x9 stimulates ERRα transcriptional activity to the same degree as PGC-1α. Equivalence of PGC-1α and PGC-1α 2x9 was also demonstrated in experiments using ERRβ and ERRγ (data not shown). ERRα transcriptional activity was unaffected by the expression of PGC-1α L2L3M, a mutant in which the leucines within the L2 and L3 motifs were changed to alanines. Thus, within the resolution of these reconstituted transcription assays, we conclude that PGC-1α 2x9 is indistinguishable from PGC-1α as an ERRα coactivator.

We next tested whether PGC-1α 2x9 could activate other NRs, in particular those known to be coexpressed with ERRα and/or subject to coactivation by PGC-1α. To this end, we compared the coactivator activity of PGC-1α or the mutants on individual receptors and cognate reporters in the presence or absence of a ligand as required. Of the receptors tested that are known to interact with PGC-1α, only HNF4α activity was enhanced to any significant degree by PGC-1α 2x9, resulting from crossreactivity of the L9 peptide, whereas the transcriptional activity of other receptors such as ERα, PPARγ, GR, LRH-1, or RXRα was unaffected (Figure 2B). Thus, by engineering the LXXLL motifs within PGC-1α, we have been able to develop a highly selective coactivator for ERRα.

Identification of ERRα Target Genes in HepG2 Cells by Using an ERR-Selective PGC-1α

A role for the ERRα/PGC-1α complex has been shown in the regulation of mitochondrial biogenesis, oxidative phosphorylation, and fatty acid oxidation in cardiac muscle and bone precursor cells (Huss et al., 2004; Schreiber et al., 2004). PGC-1α, HNF4α, and other receptors have a well-established role in the regulation of gluconeogenesis in liver cells (Puigserver and Spiegelman,

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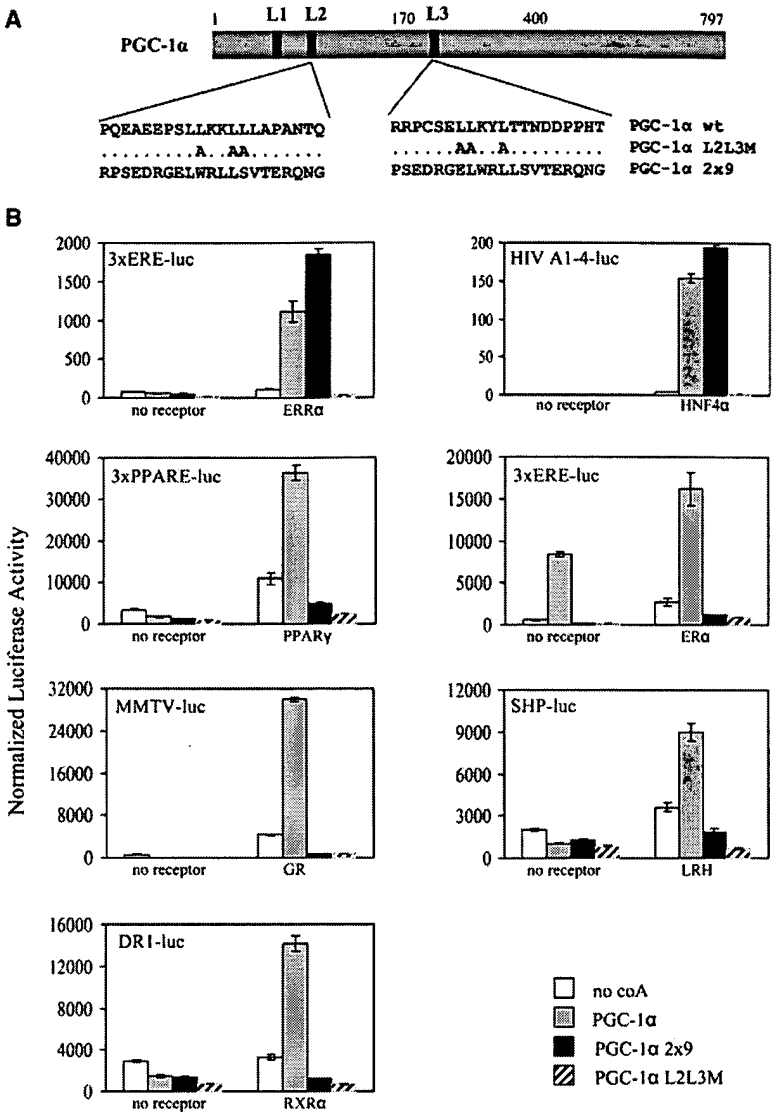

Figure 2. ERR-Selective PGC-1α Preferentially Coactivates ERR

(A) Schematic of PGC-1α LXXLL region mutants.

(B) HeLa cells were transfected with the indicated receptor, cofactor, and luciferase reporter containing the indicated response element. One-hundred nanomolar 17β-estradiol (E2), 100 nM dexamethasone, and 100 nM 9-*cis*-retinoic acid were used to activate ERα, GR, and RXRα, respectively; no hormone indicates an equivalent volume of ethanol. Results are expressed as normalized luciferase activity ± standard error of the mean (SEM) per triplicate sample of cells.

2003), but the role of ERRα in mediating energy metabolism in these cells has not been defined. As an initial step toward addressing this issue, we elected to define the target genes expressed in the hepatocellular carcinoma cell line (HepG2) that were subject to regulation by the ERRα/PGC-1α complex.

We first determined whether PGC-1α 2x9 could activate physiologic targets when transduced into target cells. PGC-1α facilitates transcriptional auto upregulation of the mRNA encoding ERRα (Laganiere et al., 2004). Thus, we measured the expression of ERRα in HepG2 cells overexpressing either PGC-1α or the 2x9 variant. As expected, ERRα mRNA and protein levels were strongly induced by both the PGC-1α and PGC-1α 2x9, but not by either of the control viruses as measured by quantitative PCR (qPCR) and immunoblot, respectively (Figure 3A). We observed no induction of endogenous PGC-1α by either the PGC-1α- or PGC-1α 2x9-expressing adenoviruses (Figure S1 in the Supplemental Data available with this article online) and proceeded to use the targeted PGC-1α to define the ERRα transcriptome in HepG2 cells.

To identify target genes induced by PGC-1α through ERRα, PGC-1α, the 2x9 variant, and the controls were independently transduced in HepG2 cells and the corresponding gene expression profiles were analyzed by using the GeneChip technology (Affymetrix, Santa Clara, CA). The resulting hierarchical clustering diagram (Figure S2) illustrates that the vast majority of genes are similarly regulated by PGC-1α and PGC-1α 2x9, differing only by degree of induction or repression. Of the genes significantly induced, 94% were induced by both PGC-1α and PGC-1α 2x9, whereas 76% of the downregulated genes were repressed by both (Figure 3B). This high percentage of similarity suggests that PGC-1α 2x9 recapitulates the activating capacities of PGC-1α and that a significant number of PGC-1α target genes in HepG2 cells are regulated by ERRα and/or HNF4α. However, quantitative differences exist between the magnitude of induction or repression by PGC-1α 2x9 compared to PGC-1α. PGC-1α induces 61% of the upregulated genes more effectively than PGC-1α 2x9, suggesting that optimal expression of these genes relies on the additional interactions by PGC-1α, in which PGC-1α 2x9 is unable

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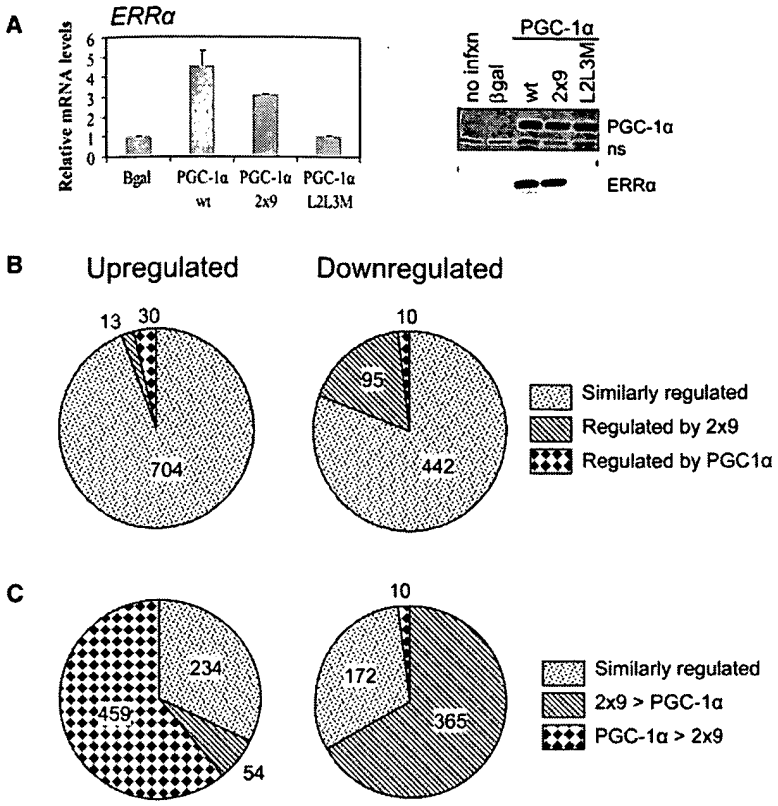

Figure 3. Regulation of Transcription by WT PGC-1α and PGC-1α 2x9 in HepG2 Cells

(A) Induction of ERRα mRNA and protein by PGC-1α constructs. Error bars indicate SEM of three biological replicates.

(B) Qualitative summary of genes significantly regulated by PGC-1α or 2x9 or both.

(C) Quantitative differences in gene regulation by PGC-1α or 2x9 based on hierearchical clustering. Expression levels from clusters compared between treatment groups using unpaired Student's t test ($p < 0.002$, where degree of induction or repression was different, $p > 0.196$ for similarly regulated clusters).

(D) Gene ontology (GO) categories that are most prominently influenced by PGC-1α and PGC-1α 2x9 related to energy metabolism. P values indicating the enrichment of these GO terms among the regulated genes were corrected for multiple comparisons.

D

GO category	*Number of genes regulated by both PGCs*	*Bonferroni-adjusted p value*
Tricarboxylic acid cycle	8	0
Oxidative phosphorylation	2	0.00934
Electron transport	22	0
Fatty acid metabolism	6	0.00799
Lipid metabolism	12	0.00384
Lipid transport	6	0.00319
Mitochondrial transport	2	0.81079
Transcription	15	1
Aminoacid metabolism	4	0.3696
Carbohydrate metabolism	10	0.07003

to participate (Figure 3C). However, 39% of the upregulated genes are induced by PGC-1α 2x9 as well or better than PGC-1α, suggesting that these genes are primarily regulated by either ERRα or HNF4α. For 67% of the downregulated genes, PGC-1α 2x9 acts as a stronger corepressor. This may result from the fact that the effective pool of PGC-1α available to ERRα is greater, as other transcription factors cannot effectively compete for binding to the modified coactivator.

Analysis of the gene ontology (GO) terms associated with the differentially regulated transcripts revealed an enrichment of terms related to energy metabolism, including oxidative phosphorylation, tricarboxylic acid cycle, fatty acid beta-oxidation, lipid metabolism, lipid transport, and electron transport (Figure 3D). Most of these pathways have been shown previously to be targets of ERRα (Huss et al., 2004; Mootha et al., 2004; Sladek et al., 1997). Other categories such as mitochondrial transport, regulation of transcription, and amino acid metabolism did not show statistical significance in number, but the biological significance of the individual genes in each class should not be discounted (Figure S3).

The PGC-1α/ERRα Complex Is a Key Regulator of Rate-Limiting Enzymes Involved in Energy Metabolism

Potential ERRα targets identified in the array described above were validated in independent experiments by using qPCR after infection with a virus expressing PGC-1α. To differentiate between ERRα– and HNF4α-dependent targets, we compared the relative levels of mRNA of PGC-1α-induced genes in cells treated with an adenovirus expressing either a control (scrambled) or ERRα

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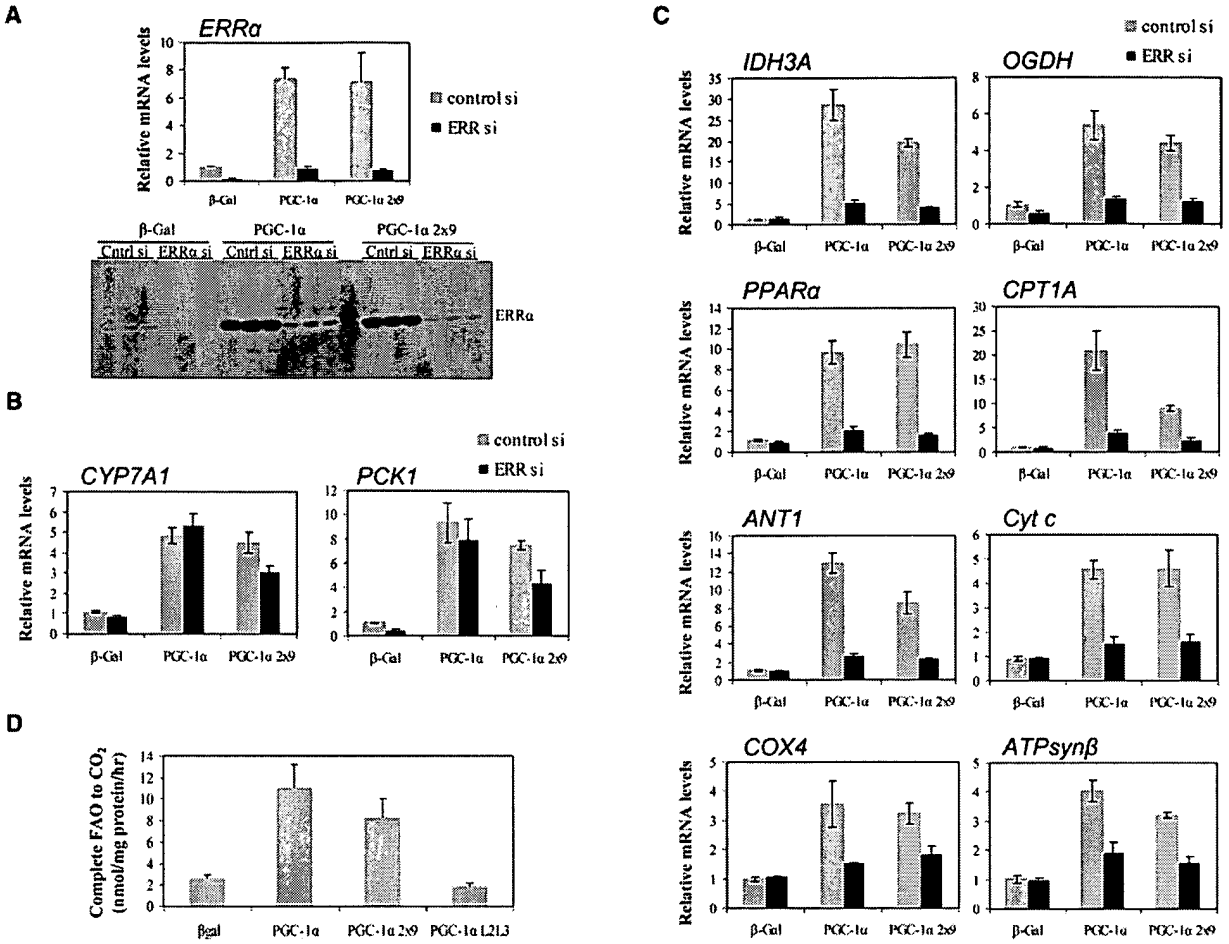

Figure 4. PGC-1α-Mediated Induction of Key Enzymes of Energy Metabolism Is Dependent on the Expression of ERRα

(A) Induction of ERRα mRNA and protein by PGC-1α or PGC-1α 2x9, and repression by the siRNA to ERRα.

(B) Genes not dependent on ERRα.

(C) ERRα-dependent genes.

Error bars in (A)–(C) represent SEM of three biological replicates.

(D) Fatty acid oxidation in HepG2 cells, normalized to protein content, with error bars indicating SEM of three independent experiments.

siRNA. The siRNA effectively inhibits the induction of ERRα mRNA and protein by PGC-1α as compared to a scrambled control siRNA (Figure 4A). Under these conditions, it was possible to distinguish known HNF4α-activated genes, such as *phosphoenolpyruvate carboxykinase 1* (*PCK1*), *cholesterol 7-alpha-hydroxylase* (*CYP7A1*), and *glucose-6-phosphatase* (*G6PD*) (Figure 4B and data not shown) from key genes of energy metabolism found to be dependent on ERRα, including *isocitrate dehydrogenase 3A* (*IDH3A*) and *oxaloglutarate dehydrogenase* (*OGDH*) of the tricarboxylic acid cycle, *peroxisome proliferator-activated receptor alpha* (*PPARα*) and *carnitine palmitoyltransferase 1A (CPT1A)* involved in regulating fatty acid oxidation, *ATP synthase β* (*ATPsynβ*, *cytochrome c* (*Cyt c*), and *cytochrome c oxidase 4 (COX4)* of oxidative phosphorylation and *adenine nucleotide translocator 1 (ANT1)* of mitochondrial transport. It is important to note that of all the genes we have studied thus far to be induced by PGC-1α 2x9 in HepG2 cells, *PCK1*, *CYP7A1*, and *G6PD* were the only genes shown to be dependent on a factor other than ERRα.

To validate this approach in other cell types, we tested induction of these ERRα target genes in AGS stomach cancer cells and U251 glioma cells and found that these genes were similarly induced by both PGC-1α and PGC-1α 2x9 in an ERRα-dependent manner (Figure S4 and data not shown). Additionally, we tested the robustness of the customizing process by exchanging a different ERRα-selective peptide, L28, for the two L9 peptides and observed that the ERRα target genes tested were induced by the PGC-1α 2x28 as well (Figure S5). Finally, to determine whether the PGC-1α 2x9 construct is functionally indistinguishable from PGC-1α in inducing one of the expected pathways, we measured fatty acid oxidation in HepG2 cells. We found PGC-1α 2x9 increases oxidation of oleic acid compared to cells expressing β-gal or PGC-1α L2L3M and recapitulates PGC-1α activity through ERRα (Figure 4D).

Discussion

It is unlikely that classical small molecule ligands will be found for all ONRs. Rather, it appears that coactivator availability or posttranslational modifications, such as phosphorylation, will emerge as primary mechanisms by which the transcriptional activity of some ONRs will

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be regulated (Hermanson et al., 2002). ERRα appears to interact primarily with members of the PGC-1 and/or p160 classes of transcription factors (Lu et al., 2001; Schreiber et al., 2003). Because these cofactors interact with several NRs and with other unrelated transcription factors, it has been exceedingly difficult to define the biological responses in cells that are attributable to individual NR-CoA complexes. With this problem in mind, we developed an approach that has allowed us to genetically isolate ERRα/PGC-1α within cells and identify target genes that were regulated by this specific NR-CoA complex. This was accomplished by engineering the LXXLL NR-interacting motifs within PGC-1α such that they would interact with ERRα at the exclusion of other NRs. Although the LXXLL motifs within the known coactivators are relatively promiscuous, we were able to identify peptides that were highly selective for ERRα by screening large peptide libraries in which the amino acids flanking the core motif were randomized. We believe that this general approach to "customize" coactivators will be applicable to other NRs, as we have found it to be relatively easy to generate LXXLL-based peptides that display a high degree of receptor selectivity. Although it is unlikely that absolute specificity of the coactivator for the targeted receptor can be achieved with this technology, this problem also exists for natural ligands of the NRs. For instance, progesterone is an effective ligand not only of the progesterone receptor but also of the mineralocorticoid and glucocorticoid receptors. Thus, confirmation of an observed biological response to a small molecule "classic" agonist or a customized coactivator will always be required. To this end, we have used siRNA in this study to confirm the involvement of ERRα in the highlighted processes. Through the use of these two technologies, comparable to the use of classical agonists and antagonists, we believe that it will be possible to probe the biology of most any transcription factor-cofactor pair.

Experimental Procedures

Plasmids

Plasmids for pcDNA3-ERRα (Zuercher et al., 2005); VP16-ERα, VP16-ERβ, VP16-RARα, VP16-RXRα, pRST7-ERα, 3xERE-TATA-luciferase, and 5xGal4-luciferase (Chang et al., 1999); LRH-1 (Safi et al., 2005); and VP16-LXR and VP16-FXR (Hall et al., 2000) were described previously. cDNA encoding ERRα and PPARγ were excised from pcDNA3-ERRα and SG5-PPARγ, respectively, and ligated into the VP16 vector (Clontech). The following were gifts: SG5-PPARγ and 3xPPARE-luciferase (T. Willson), SHP-luciferase (S. Kliewer), DR1-luciferase and RSV-RXRα (R. Heyman), HIV A1-4-luciferase and pcDNA3-HNF4α (F. Sladek), Flag-PGC-1α (B. Spiegelman), VP16-VDR (W. Pike), VP16-TRβ (D. Moore), VP16-PRA (D. Wen), VP16-GR (J. Miner), VP16-AR (K. Marschke), and VP16-RORα-LBD (A. Means).

Generation of the PGC-1α 2x9 and L2L3M Mutants

PGC-1α 2x9 was generated by excising the L2 and L3 motifs of PGC-1α and ligating in their place the sequence of the L3-09 peptide (PSEDRGELWRLLSVTERQN). This resulted in the replacement of the L2 and L3 motifs with an ERR-selective peptide and the insertion of a total of seven amino acids corresponding to restriction enzyme sites. The PGC-1α L2L3M mutant, in which the leucines were mutated to alanines, was generated by using the QuikChange II mutagenesis kit (Stratagene). All mutants were sequenced to ensure the fidelity of the resulting constructs.

Generation of Adenoviruses

Adenoviruses used to overexpress PGC-1α, PGC-1α 2x9, or PGC-1α L2L3M were generated by using the ViraPower Adenoviral Expression System (Invitrogen), purified with Adeno-x (BD Biosciences), and concentrated with a Centricon YM-50 Unit (Millipore).

The ERRα and control siRNA adenoviruses were generated as described above with the following changes: the ERRα siRNA oligonucleotides (described in Schreiber et al. [2003]) and control siRNA oligonucleotides (scrambled sequence) were ligated into pSuper (Oligoengine) and the H1-RNA promoter and the target sequence were inserted into the adenoviral backbone.

Mammalian Cell Culture, Transfections, and Adenovirus Transduction

Culture, transfection, and luciferase assays using HeLa (human cervical carcinoma) and HepG2 (hepatoma) cells were described previously (Chang et al., 1999). For assays involving hormone receptors, cells were treated with vehicle or the following concentrations of its respective hormone: 100 nM 17β-estradiol (ERα, ERβ), 100 nM progesterone (PR-A), 100 nM dexamethasone (GR), 1 μM 5α-dihydrotestosterone (AR), 100 nM 9-*cis*-retinoic acid (RARα, RXRα), 100 nM triiodothyronine (TRβ), 100 nM 1,25-dihydroxyvitamin D3 (VDR), 10 μM 22R-hydroxycholesterol (LXR), and 50 μM chenodeoxycholic acid (FXR). For transduction of protein using adenoviruses, HepG2 cells were infected at a multiplicity of infection (MOI) of 10–50 for 48–72 hr. In experiments with siRNA and PGC-1α infections, cells were infected with siRNA adenovirus 48 hr prior to a second infection with PGC-1α (or mutants).

Protein Immunoblotting

Whole-cell protein extracts were separated on an 8% SDS-PAGE and blotted onto PVDF (Millipore). PGC-1α protein was detected with a rabbit polyclonal PGC-1α antibody (Santa Cruz Biotechnology). ERRα protein was detected by using a monoclonal antibody (Gaillard et al., 2006).

Quantitative PCR

Total RNA was isolated by using the RNeasy kit with RNase-free DNase (Qiagen). One microgram of RNA was reverse transcribed by using the BioRad iScript cDNA synthesis kit. qPCR reactions (BioRad iCycler) were performed with 0.1 μl of cDNA, 10 μM specific primers, and iQ SYBRGreen supermix (BioRad). The sequences of the primers are listed in Table S1.

Fatty Acid Oxidation

HepG2 cells were infected at MOI 20 for 72 hr then incubated 3 hr at 37°C with 1 mM carnitine, 12.5 mM HEPES, 0.5% BSA, 500 μM sodium oleate, and 1.0 μCi/ml [1-^{14}C] oleic acid (MP Biomedicals). Oxidation end product ^{14}C-CO_2 released was measured as described previously (Koves et al., 2005).

Microarray Statistical and Gene Ontologic Analyses

Microarray gene profiling experiments were performed with the Human Genome U133 Plus 2.0 Array chips (Affymetrix). RNA was collected 24 hr after infection. Target preparation, hybridization to the Affymetrix HG-U133 plus 2.0 arrays, and scanning were performed by the Duke Microarray Center. The entire experiment was repeated three times. The probe intensities were extracted from CEL files by utilizing the Affymetrix Input Engine from the SAS Microarray Solution software (SAS Institute Inc., Cary, NC). After normalization, the mixed model was applied by running Mixed Model Analysis from the SAS Microarray Solution (Chu et al., 2002, 2004). Significant genes were determined by conducting t tests based on the estimated parameters from this model. The specific tests consisted of differences between β-gal control and three PGC-α variants treatment groups (WT, 2x9, and L2L3M). A Bonferroni correction was applied across all tests to control the probability of false positives to be <0.05. Enrichment in GO terms was detected by using the Onto-Express application (Draghici et al., 2003).

Supplemental Data

Supplemental Data include five figures and one table and can be found with this article online at http://www.molecule.org/cgi/content/full/24/5/797/DC1/.

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Acknowledgments

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Accession Numbers

Microarray .cel and .chp files can be found on the NURSA microarray database (http://www.nursa.org/datasets.cfm?doi+10.621/datasets.02004) and the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), accession number GSE5968.